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Structural Basis of Ciliary Movement

by Peter Satir*

All motile somatic cilia, including those of the human respiratory tract, are similar in ultrastructure in that they consist of an axenome of 9+2 microtubules surrounded by a specialized extension of the cell membrane. The axonemal elements provide the ciliary motor, which is powered by ATP hydrolysis. In respiratory cilia, mutants occur where axonemal assembly is aberrant such that the doublet attachments known as arms (Afzelius and co-workers) or spokes (Sturgess) have been shown to be missing. These mutant cilia are apparently nonmotile. In model cilia, the arms are composed of dynein, a class of ATPase isoenzymes and associated polypeptides characterized by Gibbons and colleagues. In negative stain preparations of arms, three subunits can be seen. In the presence of ATP, dynein functions with a set polarity to form transient cross-bridges that cause the microtubule doublets of the axoneme to slide relative to one another. After brief trypsin treatment, the axonemal microtubules slide almost completely apart with the arms of doublet n pushing doublet $n+1$ in a tipward direction. To produce ciliary beating *in vivo*, sliding is carefully controlled and coordinated, in part probably by the spoke system. The ciliary membrane is responsible for maintaining the appropriate levels of ATP, Mg^{2+}, and Ca^{2+} (ca. $10^{-7}M$) around the axoneme. The beat of certain cilia—e.g., L cilia of mussel gills—can be arrested by increasing axonemal Ca^{2+} concentration, for example, in the presence of the ionophore A23187 and high external Ca^{2+}. Although the net results of changes in axonemal Ca^{2+} concentration are not always complete stoppage of beat or of sliding, this ion is also part of the general behavioral control of ciliary motility.

All motile somatic cilia, including those of the human respiratory tract, are similar in ultrastructure, in that they consist of an axoneme of 9+2 microtubules, surrounded by a specialized extension of the cell membrane. The nine peripheral microtubules of the axoneme differ from other cytoplasmic microtubules in that they are doublets, composed of a complete microtubule, subfiber A, to which is attached an incomplete, shorter set of protofilaments, subfiber B. Important axonemal interconnections extend from specific points on each subfiber A: the two rows of arms, the radially directed spokes, and circumferentially directed interdoublet links. Together, these elements function as the ciliary motor, which is powered by ATP hydrolysis.

In the last decade or so, considerable advance in our understanding of the precise arrangements of these axonemal structures, together with some important analysis of their biochemistry and function, has enabled us to put together a reasonable working hypothesis of the coupling of structure and function within a cilium to produce movement. Most of the basic work on which this account is largely constructed has been derived from studies of systems other than the mammalian respiratory tract. Indeed, mammalian somatic cilia are relatively poorly studied in terms of motile mechanism and basic beat properties (*1*). Although the ciliated cells of the tracheal epithelium do not form a continuous sheet and the exact ciliary stroke of tracheal cilia is not yet understood, it is evident that these cilia are capable of moving mucus sheets in a coordinated manner to effect respiratory clearance. There is reasonable circumstantial evidence, which will be summarized here, to conclude that the basic mechanism of ciliary motion, like the ultrastructure, is similar in all 9+2 cilia, including human tracheal cilia. It remains to be seen whether the fundamental biochemical details are identical, or whether subtle but vital differences will crop up, which affect beat form and cellular control of motion. Additionally, cilia which primarily move mucus may have certain differences from cilia which move water.

Although the basic ultrastructure throughout the length of the axoneme is identical in, say, sea urchin sperm tails, *Tetrahymena* or mussel gill cilia, and in tracheal cilia, differences do occur at either end, so that the origins, overall lengths and attachments of the individual doublet microtubules are not the

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same. For example, a sperm tail may be 50 μm long, a tracheal cilium only one tenth to one-fifth of that length; one cilium may taper, another end in a blunt, rounded end, and so on. One comparison is shown in Figure 1. These differences again are thought to modify the actual form of beat, while the basic motile mechanisms remain reasonably constant.

Sliding Microtubules in Ciliary Motion

Ciliary motion is based on the sliding of the doublet axonemal microtubules. Several major aspects of this sliding microtubule mechanism are now well established.

The axoneme alone is responsible for ciliary motility. Detergent-treated models of cilia beat normally in appropriate solutions containing 1*mM* concentrations of Mg^{2+} and ATP. Detergent treatment destroys the integrity of the ciliary membrane but causes no substantial change in the arrangement of axonemal components. In the intact cell, the ionic environment of the axoneme, and concentrations of ATP and divalent cations are maintained and regulated by the ciliary membrane. The presence of the membrane permits cellular control of axonemal function.

The doublet microtubules of the axoneme slide relative to one another without measurable contraction (*3*) during bend production in a ciliary stroke. In a recent test of the geometrical predictions of this statement, Shingyoi et al. (*4*) pipetted ATP onto local regions of Triton-extracted sea urchin sperm whose heads had been attached to a micropipet. This induced local equal and opposite bends of the axoneme, without change in head or tail position, which conforms exactly to the necessary constraints of a sliding system. Further, Triton-treated axonemes exposed briefly to trypsin, then washed and placed in ATP, do not beat but instead disintegrate by telescoping apart (*5*). Here sliding of individual microtubules is directly observed. Figure 2, taken from the work of Satir and Sale (*6*) shows an axoneme prepared for electron microscopy after such sliding has taken place. Sliding in the trypsin-treated axonemes is relatively isotropic — i.e., at least seven and possibly all nine doublets are capable of sliding. It is also unidirectional. If doublets are numbered 1-9 in the conventional way, the doublet $n+1$ is always displaced tipward relative to doublet n. Lastly, sliding in such cases is uncoupled from bend generation and bend propagation and largely unconstrained: final extension can be many tens of micrometers longer than the original axoneme. Only one constraint seems to limit sliding in the preparations: some overlap of doublets is always necessary, for

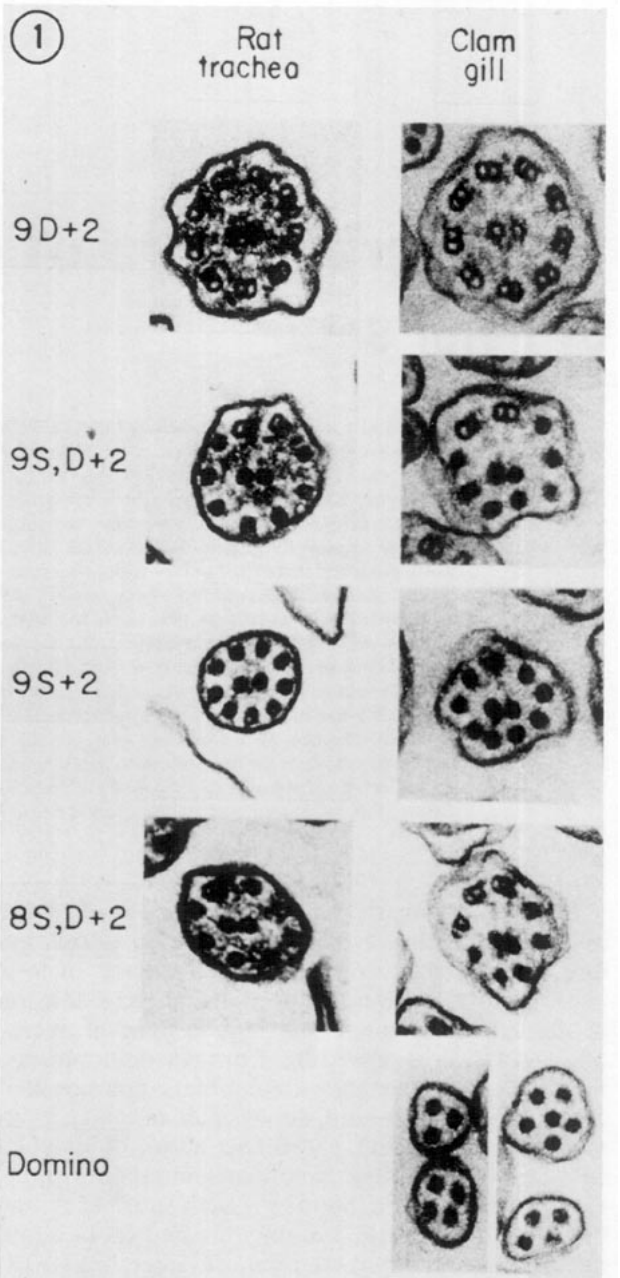

FIGURE 1. Comparison of ultrastructure of the axoneme (9D+2) and tip configurations of mammalian respiratory and molluscan gill cilia. The finding of 9S+2 and 8S, D+2 tips in separate cilia is indicative of relative sliding of doublets. Details of nomenclature and interpretation are given elsewhere (*2*). The tracheal cilia end in a rounded tip and the doublet microtubules are roughly all of equal length; this is not so for the gill cilia, whose tapering tip results in the domino cross-section. × 92,500.

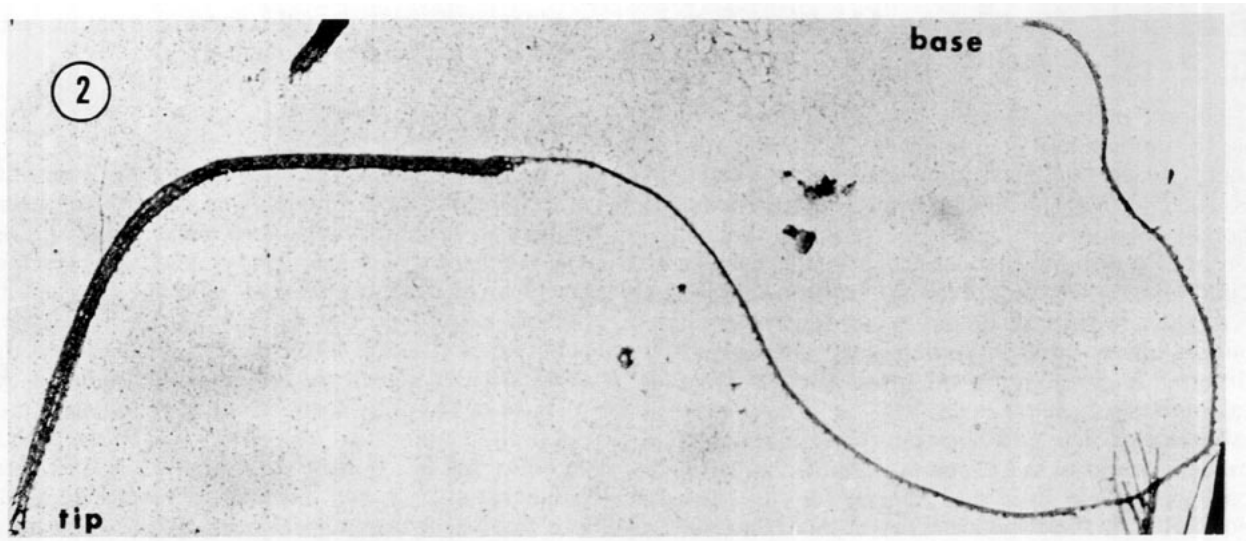

FIGURE 2. Sliding microtubules in a trypsin-treated ciliary axoneme to which ATP is added. For preparative details, see Satir and Sale (6). × 18,800.

reasons dealt with in the next paragraph.

Active sliding is generated when the arms attached to subfiber A of each doublet (i.e., doublet n) also bind to subfiber B of the adjacent doublet ($n+1$) to push that doublet. The generation of active force in this system is accompanied by ATP dephosphorylation. Because the arm action is repetitive and cooperative, we can define an arm cycle, which is discussed further below. Presumably, sliding in trypsin-treated axonemes stops when the number of active arms is no longer sufficient to move the doublet microtubules. At that time, evidently, a small amount of overlap between adjacent doublets still remains (Fig. 2). Individual doublets appear inactive when completely separated from the axoneme.

Active sliding and bending are two separable systems; the latter relies on coordination and restriction of sliding, and is trypsin-sensitive. In the intact (not-trypsin-treated) axoneme, microtubule sliding rarely exceeds 0.3-0.4 μm maximum displacement, that is, only a few percent of total possible sliding. Further, sliding is not unidirectional. This has been interpreted to mean that during beat, doublets slide asynchronously such that the active sliding of doublets 1-4 produces the ciliary effective stroke, while doublets 6-9 are passively moved, and alternatively, active sliding of doublets 6-9 produces the recovery stroke, while doublets 1-4 are passively moved (*6*).

Much less is known about the conversion of active sliding into bending and about mechanisms of bend propagation than about sliding itself. The radial spokes, the interdoublet links, and perhaps the arms themselves presumably form part of an elaborate mechanical feedback system within the axoneme that switches sliding on and off appropriately. The spokes, in particular, interact with central sheath projections in a highly circumscribed manner, such that as bends develop at the base of a straight axoneme during a beat, the spokes become tilted in a manner consistent with attachment of the spoke head as a method of constraining further sliding and producing bending (*7*). It seems likely that the spoke-central sheath interaction is central to the local conversion of sliding to bending, or at least to the trypsin-sensitive system which causes the conversion. Spokes, especially the spoke heads, and interdoublet links appear to be the principal structures digested by trypsin under conditions where sliding restraints disappear. Further, mutants of motile *Chlamydomonas* lacking either structural component of the spoke-central sheath complex are paralyzed despite the presence of the intact sliding system (*8*).

Calcium ion concentration surrounding the axoneme provides a usual control of ciliary activity. Cilia beat normally in the presence of axonemal free $Ca^{2+} < 10^{-7}M$. In many organisms, alteration of axonemal free Ca^{2+} to greater than $10^{-6}M$ causes major alteration in ciliary behavior, including complete cessation of motion (*9*). However, the trypsin-resistant sliding of ciliary axonemes seems insensitive to changes in Ca^{2+} that physiologically account for ciliary arrest (*10*), so that it seems probable that the site of Ca^{2+} control resides in the trypsin-sensitive feedback system responsible for bend generation and propagation. In organisms where increasing Ca^{2+} does not cause complete arrest, it is the mode of bend generation and propagation that is usually altered, while sliding must obviously continue.

Evidence for These Mechanisms in Respiratory Cilia

Ciliary malfunction has recently been convincingly documented in man (*11*). Immotile cilia in males results in a syndrome, formerly known as Kartagener's triad, which couples infertility due to nonmotile sperm to respiratory disease (sinusitus, bronchiectasis) due to nonfunctional respiratory cilia. (The third aspect of the syndrome described by Kartagener, situs inversus, is not always present, and its link to ciliary function is still obscure). The diseases responsible for the syndrome are congenital, and affect females as well as males. Several laboratories have now shown ultrastructural defects present in sperm tail axonemes and somatic cilia in patients whose cilia and sperm are functionally nonmotile. In the original cases, the cilia lacked arms (*11*). Sturgess et al. (*12*) have now described a second variant, where the cilia possess arms but lack radial spokes. From the complexity of ciliary structure and assembly, it is quite likely that other variants will be found, including those where the ultrastructural defect is not apparent (*13*). Although the biochemistry remains to be worked out, the mutant classes in humans resemble those in lower organisms where the rationale for ciliary paralysis has been defined. Presumably, in armless axonemes the doublets are incapable of sliding. It would be interesting to know whether the doublets of the spokeless human axonemes would slide upon appropriate treatment; presumably, these axonemes are defective in the conversion of sliding into propagated bending. Despite this impressive circumstantial evidence for mammalian cilia, there is as yet no direct evidence regarding the mechanism of motion. However, sliding of axonemal microtubules in mammalian sperm has been demonstrated (*14, 15*).

In 1967, Spock reported (*16*) that addition of serum from cystic fibrosis (CF) homozygotes or heterozygotes to excised rabbit ciliated tracheal epithelium which was beating in a coordinated manner resulted in "ciliary dyskinesia." Although much controversy has been generated regarding the existence of specific CF factors, and dyskinesia has never been adequately described in a quantitative manner, ciliary beat clearly looks more or less coordinated under different conditions. Bogart et al. (*17*) report a CF-like dyskinesia when the divalent cationophore A23187 is added to the medium bathing the tracheal epithelium. Can it be that mammalian ciliary axonemes are also responsive to changes in Ca^{2+} and that dyskinesia is the equivalent of ciliary arrest? If this were so, agents such as cigarette smoke, industrial pollutants, immune complexes etc. which might alter the cell or ciliary membrane could all act to produce dyskinesia via a final common pathway involving increased free axonemal Ca^{2+}.

Dynein Arm Cycle

The aspect of the mechanism of ciliary motility that is proving most amenable to analysis at present is the arm cycle that produces microtubule sliding. The biochemistry of the arms is unsettled in detail, but clear in general principle. The arms are multiprotein structures, whose main functional components are a class of ATPase isoenzymes called dyneins (*18*). In *Tetrahymena* ciliary axonemes, the ATPase of the arms seems to correspond to that of sea urchin sperm tails, dynein-1 (*19*). Dynein-1 can be extracted in a form that has latent ATPase and sediments at 21 S, and this may represent the intact arm that is the moving cross-bridge between adjacent axonemal doublet microtubules and that generates sliding. The 21 S particle is apparently composed of three subunits of about 330 kdaltons and one each of subunits of 126, 95, and 77 kdaltons. It is not yet known whether the inner and outer rows of arms are exactly identical in structure or in composition.

In negative stain, the subunit construction of the arms is visible (*20*). The arms have an overall tripartite construction; in at least one position they are slightly tilted (ca. 40°) towards the base of the subfiber A to which they are attached (doublet n). In the absence of ATP and the presence of $2mM$ Mg^{2+}, both inner and outer arms attach to the subfiber B of doublet $n+1$, forming bridges between doublets as is illustrated in Figure 3*a*. This is known as the rigor position (*21*). Such rigor bridges can be plasticized by the addition of ATP, as in Figure 3*b* [see also Zanetti et al. (*22*)].

In trypsin-treated axonemes, presumably held together largely by rigor arms in the absence of ATP, upon ATP addition, as the arms are plasticized, the axonemes fall apart, with or without concurrent microtubule sliding (*10*). This step is evidently intrinsic to the dynein-microtubule interaction per se. For example, Takahashi and Tonomura (*23*) have recombined dynein arms to doublets from which such arms had previously been extracted. In the presence of Mg^{2+} with no ATP, in a state equivalent to rigor, the arms rebind to and decorate either subfiber A and to subfiber B. Upon addition of ATP, equivalent to plasticizing the rigor bridge, the arms detach from subfiber B exclusively.

It is postulated that sliding of the doublet microtubules is accomplished by successive detachment-reattachment of the dynein arms in this way, each detachment requiring binding of new ATP, which is then hydrolyzed at some point in the subsequent

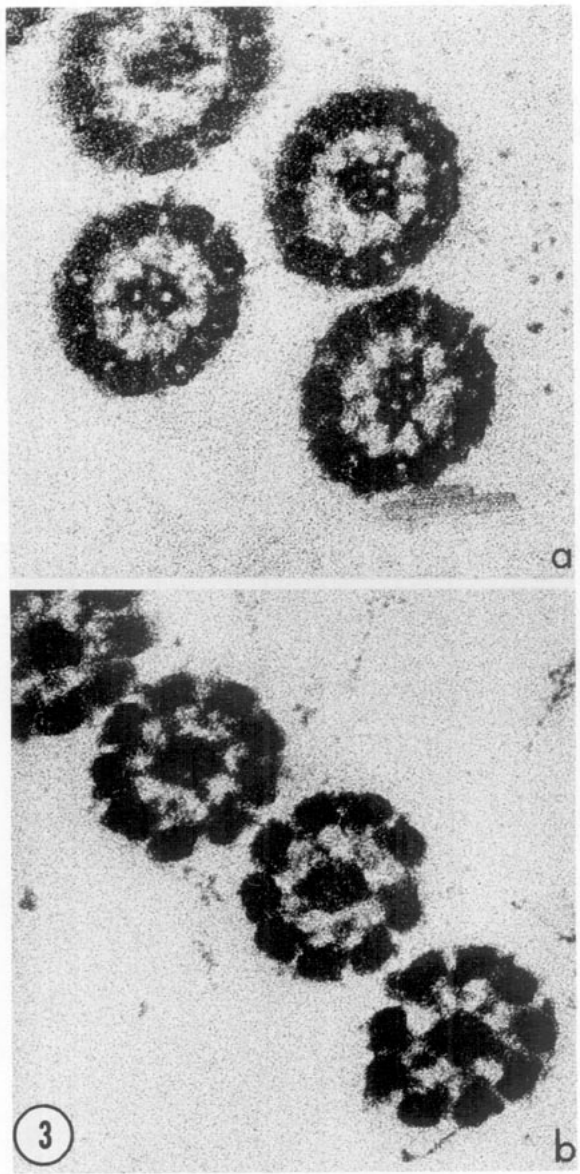

FIGURE 3(*a*). Triton-treated axonemes before ATP addition. All doublets are bridged by rigor arms. × 126,000. (*b*). After ATP addition. Rigor arms are plasticized, so that the doublets are no longer completely attached to one another. × 126,000.

cycle, providing the energy for eventual directional force generation. There is little experimentation and no general agreement yet on the exact details of the cycle, but one possibility which combines the known structural information with a plausible, though highly speculative, enzymology is shown in Figure 4. The enzymology is simply drawn as a parallel to the work of Taylor and his colleagues (25) on the course of actin-myosin kinetics. For cilia, it is known that vanadate is a specific inhibitor of dynein-ATPase activity and of doublet sliding, but probably

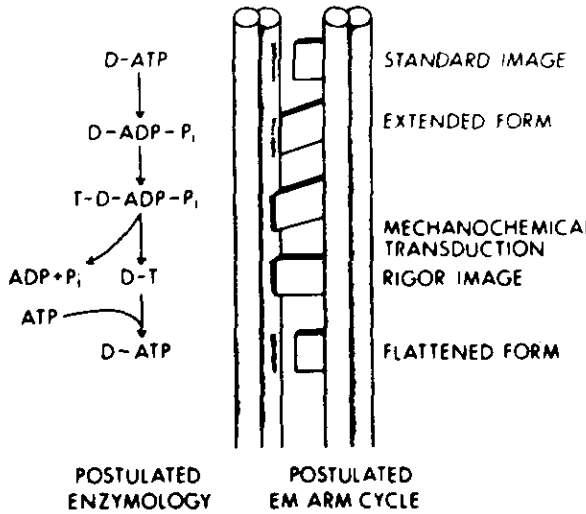

FIGURE 4. Hypothetical dynein arm cycle, from Satir (24). Successive stages of one complete cycle are shown from top to bottom. At each level, the postulated enzymology is correlated with the postulated arm morphology. The hatched vertical line along subfiber B of doublet $n+1$ as diagrammed indicates hypothetical arm attachment site.

not of doublet detachment (26). This may prove to be an important tool in clarifying the point in the cycle at which ATP is actually hydrolyzed. Eventually, the combination of such approaches to the biochemistry, physiology and morphology of the dynein arm and to other axonemal structures should yield a fundamental understanding of the underlying mechanochemical transduction events in ciliary motion.

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